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Antioxidant efficacy and cytotoxicity of ethanol extract of *Clerodendrum infortunatum* against different cell lines

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Clerodendrum infortunatum belongs to the Lamiaceae family and is a perennial shrub. It is widely known for their important medicinal values among the Mizo tribe. In the present study, the preliminary phytochemical screening, quantification of phenols, flavonoids and alkaloids, antioxidant activities by DPPH, O^{2-} and ABTS assays and cytotoxicityby MTT assay against AGS (gastric cancer), HeLa (cervical) and HT-29 (colon) cell lines compared with normal cell line (Chang liver) were performed. Furthermore, the GC-MS profiling was also conducted. The results imply the presence of saponin, alkaloid, cardiac glycoside, phenol and flavonoid. The quantification shows that phenol content (64.35 mg/ g) was highest followed by flavonoid (61.93 mg/ g) and alkaloid (13.33 mg/ g). Its scavenging efficiency against DPPH with IC_{50} value was 47.99, against O^{2-} with IC_{50} was108 µg/mL and against ABTS cations with IC_{50} was 50.05 µg/mL, respectively. The ethanol extract exhibited a maximum cytotoxicity against HeLa with IC_{50} value of 53.55 µg/mL, AGS with IC_{50} value 82.44 µg/mL and HT-29 with IC_{50} value of 142.2 µg/mL. However, the extract showed comparatively less toxicity against normal cell lines. Moreover, 14 active compounds were confirmed in the GC-MS analysis of the extract. HPLC study also infers the occurrence of the flavonoids rutin and quercetin. Therefore, the results of *C. infortunatum* ethanolic extract clearly specified that it has a very high antioxidant activity as well as cytotoxic properties; which proved that this ethnomedicinal plant can be used as an alternative agent to treat a variety of illnesses.

Keywords: Antioxidant, Cell lines, *Clerodendrum infortunatum*, GC-MS, Mizoram

The use of plant based drug is slowly rising in popularity since the past decade in both the developing and developed countries. These herbal drugs are more readily available for consumers as compared to the synthetic drug since they are easily accessible in many shops not only in drug stores. It is believed that herbal medicine is used by 80% of the world's population[1-3]. The people of Mizoram rely largely on herbal drugs which have been inherited for generations traditionally. It is through nature, especially the plants that the human race obtained many drugs or chemicals for the treatment of different ailments. Many drugs which play a crucial part in modern medical science are prepared from plants[4]. Since cancer has become a significant concern in public health worldwide, vinblastine and vincristine were among the earliest cancer treatments to enter clinical trials which were extracted from the plant, *Catharanthus roseus*[5]. They are frequently used in amalgamation with other chemotherapeutic medications to treat a variety of cancers[6]. The pursuit for newer anti-cancer drugs perseveres due to the numerous adverse effects of the currently used drugs especially the synthetic ones besides the high chance of tumour recurrence[7]. Besides antineoplastic and other activities, medicinal plants become more important sources for antioxidant compound[8]. The presence of compounds like phenolic and flavonoids possesses high antioxidants activities that can inhibit the free radicals to prevent several harmful ailments[9-11].

Clerodendrum pertains to the category family Lamiaceae and is also known as glory bower, bag flower, and bleeding-heart[12,13]. It is indigenous to tropical regions of Africa and southern Asia[14]. *Clerodendrum* are often known to have pesticidal properties[15], and various *Clerodendrum* species are employed in the discipline of traditional medicine for curing different ailment such as inflammation, toothache, cold, hypertension, asthma, furunculosis, rheumatism, anorexia, leprosy, dysentery, arthrophlogosis, mammitis and leucoderma in many parts of the world[16-19]. It is well explored in scientific study relating to anticancer properties including human cells

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such as T47D (Breast), PC-3 (prostate), A549 (lung) and HCT-116 (colon)[20,21]. *Clerodendrum* were known to constitute of scutellarin, luteolin-7-3 glucuronide, apigen, stimasterol, D-glucose of sitosterol, queretonic acid, baicalin, oleonolic acid, serratagenic acid, D-mannitol and α-spinasterol in various parts such as leaf, bark and root[22]. In the past few decades, many researchers studied the genus *Clerodendrum* for its high impact of medicinal efficacy. *Clerodendrum serratum* Linn. leaf extract was reported for *in vivo* and *in vitro* studies of its anti-anthelmintic activity, hepatoprotective, anti-oxidant, anti-inflammatory, analgesic and antiasthmatic[23,24]. The species such as *C. philippinum, C. splendens* and *C. viscosum* were evaluated for antimicrobial, antioxidants, larvicidal and pupicidal activities[25-27].

Moreover, *Clerodendrum colebrookianum* is a common vegetable among local people and as herbal medicine for treating hypertension, diabetes and colics in infants particularly in Mizoram. However, *C. infortunatum* is not considered edible but used as medicinal purposes including treatment for scabies, as antidandruff agent, some skin diseases[28]. *C. infortunatum* is not yet explored scientifically in this particular region though it is used in various medicinal purposes by the local people. Therefore, the purpose of this study is to assess the antioxidant property and cytotoxicity of ethanolic extract of *C. infortunatum in vitro*.

Materials and methods

Chemicals and reagents

All the chemicals and reagents Quercetin, Folin-Ciocalteu, Gallic Acid, Aluminium chloride ($AlCl_3$), Hydrochloric acid, Nitro blue tetrazolium(NBT), Ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT), Ethanol, Dimethyl sulfoxide (DMSO), Bromocresol green solution (BCG), Dulbecco's Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), trypsin and Ethylene diamine tetra acetic acid (EDTA) used in this study are analytical grade and obtained from Merck Company, Germany.

Collection of Plant materials

The *Clerodendrum infortunatum* L. was collected from Tanhril Village, Aizawl, Mizoram. The specimen was assigned an accession number 564 after the proper identification was made by Prof. H. Lalramnghinglova, a known botanist as well as the Coordinator of Mizoram University Herbarium (it is an authorized herbarium in the university/state). Further, the specimen as a herbarium was submitted in this Mizoram University Herbarium for future reference.

Preparation of extracts

The freshly cleansed *C. infortunatum* leaves were air-dried for 72 h at room temperature before being ground in a grinder. The powder (250 g) was extracted for 24 h with 500 mL ethanol in a glass conical flask using a shaker at 25°C and filtered. Using a rotary evaporator under low pressure, the crude extract was concentrated at 40°C. The extract was preserved after drying and stored at 4°C in airtight containers until needed.

Preliminary phytochemical screening

The phytochemicals screening of the plants extract was done using standard protocols[29-31].

Determination of the total phenolic, flavonoids and alkaloids content

Total Phenol content

Folin-Ciocalteau reagent was used to figure out the total phenols[32]. 500 µL of ethanolic extract of *C. infortunatum* leaves was mixed with Folin-Ciocalteau solution (5 mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 mL, 1 M). A UV/Visible double beam spectrophotometer at 756 nm was used to find out the phenol concentration after 15 min of incubation. Gallic acid equivalent was used for the calculation of the phenol content (mg/g dry weight), which is a widely used standard.

Total flavonoids content

Flavonoids quantification was done following the aluminium chloride method[33]. 0.5 mL (1 mg/mL) of *C. infortunatum* extract was combined with 1.5 mL methanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL distilled water in various concentrations. It was then kept at room temperature for 30 min. The quantity of flavonoids was estimated by measuring the optical density at 415 nm using a UV/Visible spectrophotometer.

Total Alkaloids content

For evaluation of the total alkaloid content, Bromocresol green method[34] was used. A solution (BCG) was mixed with 1 mL of *C. infortunatum* extract and the mixture was agitated and complex formed was extracted with 5 mL of chloroform. Absorbance was taken at 415 nm against blank. The solutions were stable for 2 h. Atropine was used as positive control and extract was calculated against blank.

Antioxidants Activities

DPPH scavenging activity

The DPPH free radical scavenging activity was measured using the Leong and Shui method[35], with slight modifications. In 1 mL solution of 0.1 mM DPPH, 0.5 mL of each concentration of the *C. infortunatum* extract was added. The absorbance was measured after it was thoroughly mixed and incubated for 30 min (523 nm). The OD of the plant extract and the control were compared. The result was expressed as a percentage and was calculated using the following formula:

$$\% \text{ Scavenging} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

where, A_{sample} is the absorbance of the test sample and $A_{control}$ is the absorbance of the control.

ABTS cation scavenging assay

The *C. infortunatum* extract ABTS cation scavenging activity was assessed using the decolorization method[36], in which the plant extract transformed the green colour of an unstable ABTS solution to colourless. The formula used to compute the percent suppression of absorbance at 734 nm is:

$$\text{ABTS scavenging effect } (\%) = \frac{(AB - AA)}{AB} \times 100$$

where, AB is absorbance of ABTS radical + methanol; AA is absorbance of ABTS radical + sample extract/standard. Trolox was used as positive standard substance.

Superoxide anion scavenging assay

This Superoxide anion scavenging activity was determined with slight modifications[37]. To the mixture, 0.2 mL of NBT (1 mg/mL of solution in DMSO), 0.6 mL *C. infortunatum* extract, 2 mL of alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 0.1 mL H_2O) were added in a final volume of 2.8 mL. The ascorbic acid was used as a positive control.

Cell lines and Culture medium

HeLa (cervical cancer), HT-29 (colon cancer), AGS (gastric cancer) cell lines were obtained from NCCS, Pune, India. Cell lines were grown at 37°C in DMEM with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (5 µg/mL) in a humidified environment of 5% CO_2 until 85% confluency. Trypsin solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS) was used to separate the cells.

Cytotoxicity

The cytotoxic activity of the plant extract was determined by reduction of metabolically active cells through mitochondrial enzymes[38] on HeLa, HT-29 and AGS cell lines. Approximately, 1×10^4 cells per well were seeded in 96-well plates. The cells were allowed to attach to the bottom of flask prior to the experiment. After 16 h incubation period, cells with 70-80% confluency were treated with *C. infortunatum* extracts at various doses and incubated for 72 h. The cells were then given 10 µL of MTT (5 mg/mL) solution and the plate was placed to a cell incubator for another 4 h. At a wavelength of 550 nm, the proliferating cells were determined using a Spectramax M2 Microplate Reader (Molecular Devices, USA). The untreated wells were taken for measuring the relative viability, where cells were found 100% viable. The results are expressed as mean values (±SD) of three repeats.

Identification of bioactive compound using GC-MS analysis

The GC-MS analysis was carried out using a Clarus 690 Perkin/Elmer (Auto system XL) Gas Chromatograph with a mass detector Turbo mass gold Perkin Elmer Turbomass 5.1 spectrometer and an Elite 1 (100% Dimethyl poly siloxane) capillary column measuring 123.5 M × 678 M. The instrument was fixed at an initial temperature of 40°C ramp 5°C/min to 115°C, hold 5 min, ramp 5°C/min to 140°C, hold 5 min, ramp 2°C/min to 210°C, hold 8 min, and maintained at this temperature for 3 min. At the end of this period, the oven temperature was increased up to 250°C, at the rate of 5°C/min, and maintained for 9 min. The injection port temperature was maintained at 250°C, while the flow rate of Helium was kept at 1.5 mL/min. 70 eV was used as the ionisation voltage. The samples were injected in a 10:1 split mode. The scan range for mass spectral scanning was set to 500-800 (m/z). The temperature of the ion source was ensured at 230°C, while the temperature of the interface was kept at 240°C. The MS start time was 3 min, and end time was 75 min with solvent cut time of 3 min. The spectrums obtained of volatile compounds detected the through GC-MS were compared and matched with NIST 17 online library Ver. 2.3[39].

HPLC chromatogram analysis

The presence of bioactive compounds were quantified using Shimadzu Instrument (Shimadzu Corp, Kyoto, Japan) coupled with a diode array detector (DAD, SPD N 20A) and C18 column (5 µM;

4.6×250 mm). The analytical procedure was executed by applying the chromatographic conditions[40]. The gradient system began with a concentration of 100% solvent A at 0.1 min, then progressively increased the concentration of solvent B to 35% in 25 min, 50% in 45 min, and eventually 100% in 65 min. The standard compounds quercetin and rutin were dissolved in sterile water and filtered by PVDF (0.45 µM) and a volume of 20 µL was eluted. The solvent system of HPLC grade H_2O (solvent A) and H_2O: C_2H_3N: CH_3COOH (48:51:4 *v/v*) (solvent B) were employed as mobile phase. The flow rate was kept at 1 mL/min and 20 µL of the sample was injected for the analysis. The reference compounds such as quercetin and rutin were analyzed discretely to obtain the retention periods.

Table 1 — Preliminary phytochemical screening of ethanol extract of *Clerodendrum infortunatum* leaves

Phytochemicals	Test result
Saponins	+
Tannins	-
Cardiac Glycosides	+
Flavonoids	+
Alkaloids	+
Phenols	+

Linearity

The acquisition of linearity was acquired by repetitive elution of various concentrations of standards (quercetin and rutin). Analytical curves were obtained for every compound compared with their correlation in the peak area and the concentration using linearity.

Statistical analysis

The statistical analysis was carried out using Microsoft excel 2010, Washington, USA. The IC_{50} was calculated using GraphPad Prism 7 software, California, USA. The results are expressed as mean ± standard error of the mean (SEM).

Results

Preliminary phytochemical screening

According to the findings, the ethanol leaf extract of *C. infortunatum* showed the presence of saponin, alkaloid, cardiac glycosides, phenol and flavonoid but tannin was found to be absent (Table 1).

Determination of the total phenolic, flavonoids and alkaloids content

The *C. inforunatum* contain high phenol 64.35 mg/g extract followed by flavonoid 61.93 mg/g extract and alkaloid 13.33 mg/g extract expressed as gallicacid, quercetin and atropine equivalents, respectively (Fig. 1).

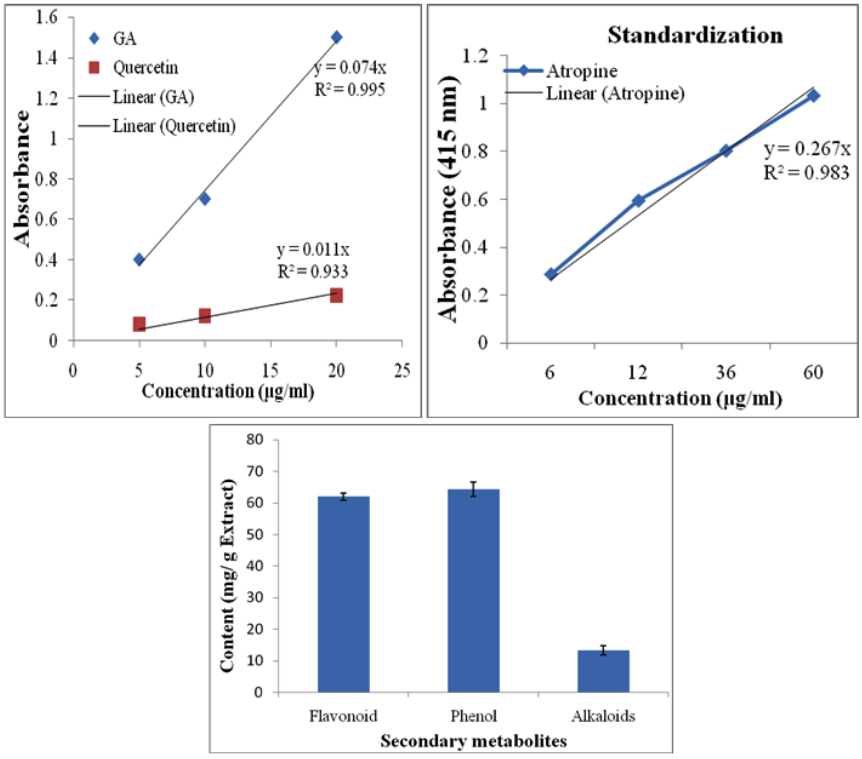

Fig. 1 — The calibration and quantification of secondary metabolites content of *C. infortunatum.* (A & B) Standardization of phenol and flavonoids; and (C) Quantification of secondary metabolites. The data present as mean ± SEM, n=3

Antioxidants Activities (DPPH scavenging activity, ABTS cation scavenging assay, Superoxide anion scavenging assay)

The antioxidant activity was assessed by the scavenging ability of DPPH, ABTS and Superoxide free radicals. The percentage inhibition of ABTS at 80 µg/mL sample gives the highest inhibition (90.14%) with an IC_{50} value of 50.05 followed by DPPH at 80 µg/mL inhibition 86.90% with IC_{50} of 47.99 and superoxide at 100 µg/mL sample shows 54.02% inhibition with IC_{50} of 83.57, respectively, (Fig. 2).

Cytotoxicity

The cytotoxicity of *C. inforunatum* extract was carried out on different cancer cell-lines such as HeLa, AGS and HT-29 along with chang liver (normal cell line). The result exhibited concentration dependant toxicity where the IC_{50} against HeLa is 52.48 followed by AGS (82.44) and HT-29 (142.2). However, the normal cell line (chang liver) showed less toxicity against plant extract except HT-29 where inhibitory concentration was found to be increased (Fig. 3).

Identification of compound using GC-MS analysis

The GC-MS analysis of the *C. inforunatum* showed the presence of 14 active compounds. The identified compounds were as follows, 9,12,15-octadecatrienoic acid(Z,Z,Z)- (3.864%), Ethyl 13-Methyl-Tetradecanoate (3.668 %), neophytadiene (3.372%), tridecanoic acid,12-methyl-Methyl ester (2.588%), methyl 11-Mrthyl-Dodecanoate (1.546%), Phenol,4-(-Methylpropyl) (1.422%), Bis (1,2,2-Trimethylpropyl) Methylphodhonate (1.285%), Benzofuran,2,3-Dihydro (0.909%), Phosphinothioic fluoride (0.883%), Imidazole,4-fluro- (0.604%), Oleic acid (0.595%), 3,7,11,15-Tetramethyl-2-Hexadecen-1-ol (0.456%), 4-cyclopentene-1,3-dione (0.441 %), Cyclobutanethiol (0.373%) (Table 2 & Fig. 4).

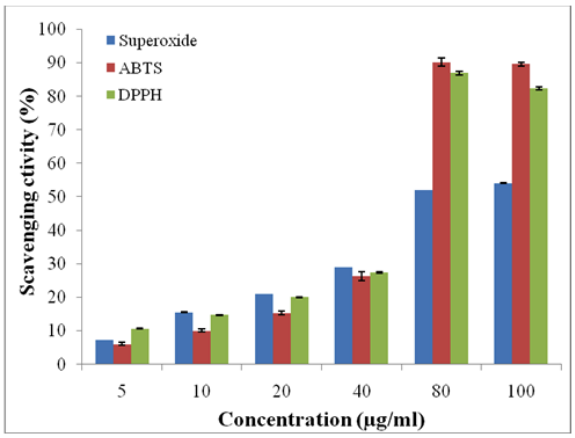

Fig. 2 — The free radical scavenging activity of *Clerodendrum infortunatum* extract. The data measured as percentage inhibition and represent as Mean± SEM, n=3

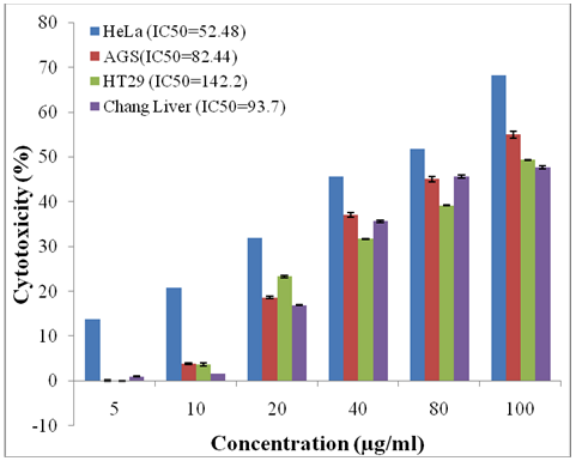

Fig. 3 — The cytotoxicity of *Clerodendrum infortunatum* extract against AGS, HeLa, HT-29 and normal cell line. The data measured as percentage inhibition and represent as Mean± SEM, n=3

Table 2 — GC-MS profiling of the identified compound from the ethanol extract of *Clerodendrum infortunatum* leaves

Peak	RT	Molecular weight	Molecular formula	Area %	Name of the compound
1	7.129	86.07	$C_3H_3FN_2$	0.604	Imidazole,4-fluro-
2	7.939	88.17	C_4H_8S	0.373	Cyclobutanethiol
3	11.171	96.08	$C_5H_4O_2$	0.441	4-cyclopentene-1,3-dione
4	18.492	306.21	$C_{10}H_9F_6PS$	0.883	Phosphinothioic fluoride
5	20.454	-	-	1.233	Unknown
6	23.909	120.15	C_8H_8O	0.909	Benzofuran,2,3-Dihydro
7	24.521	264.34	$C_{13}H_{29}O_3P$	1.285	Bis(1,2,2-Trimethylpropyl) Methylphosphonate
8	27.676	225.28	$C_{12}H_{19}NO_3$	1.422	Phenol,4-(-Methylpropyl)
9	55.124	278.5	$C_{20}H_{38}$	1.557	Neophytadiene
10	57.360	296.5	$C_{20}H_{40}O$	0.456	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol
11	59.980	228.37	$C_{14}H_{28}O_2$	1.546	Methyl 11-Methyl-Dodecanoate
12	62.745	282.5	$C_{18}H_{34}O_2$	0.595	Oleic acid
13	63.373	270.5	$C_{17}H_{34}O_2$	3.668	Ethyl 13-Methyl-Tetradecanoate
14	68.468	298.9	$C_{18}H_{31}ClO$	0.467	9,12-octadecadienoylchloride, (Z,Z)-
15	69.823	242.4	$C_{15}H_{30}O_2$	2.588	Tridecanoic acid,12-methyl-Methyl ester

Table 3 — HPLC method validation of the standards

Standards	RT	R^2	Formula (Y=m(X)+C)	LOD (µg/L)	LOQ (µg/L)	Accuracy (%)	Peak purity index
Rutin	6.603	0.99	Y = 2283X - 915	0.73	0.3	100.3	0.78091
Quercetin	8.470	0.99	Y = 3569X - 147	0.24	0.15	103.1	0.8385

Linear calibrations curves were not forced to zero; RT-Retention time; R^2- Coefficient correlation; LOD-Limit of detection; LOQ-Limit of quantification

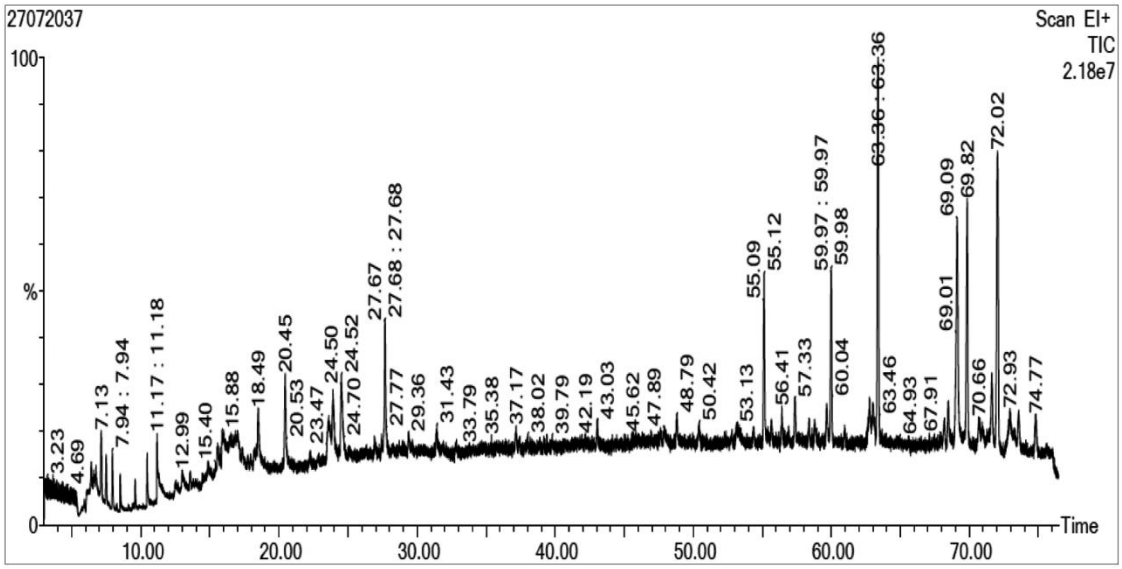

Fig.4 — GC-MS chromatogram of the ethanol extract of *Clerodendrum infortunatum*

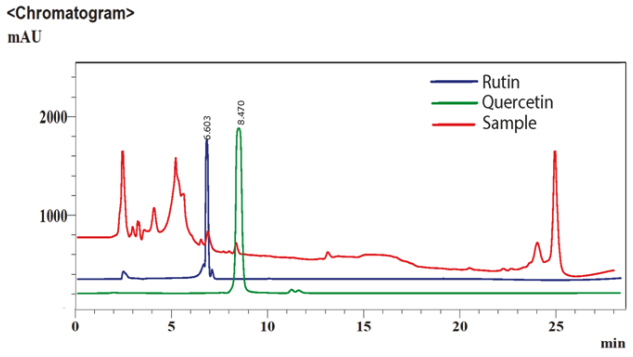

Fig. 5 — Detection of bioactive compound of *Clerodendrum infortunatum* extract by HPLC method

The HPLC analysis of *C. inforunatum* revealed the presence of rutin at the retention time of 6.603 and quercetin at the retention time of 8.470 (Table 3 & Fig. 5).

Discussion

Herbal medication is still practiced in many developing countries and is believed to be the cheapest and safest medication and promotes healthier living as well[41]. This could be the reason for the boom in sales of herbal medicines which constitutes a significant share of the global drug market[42,43]. Therefore, it was decided to investigate the phytochemical constituents of *C. infortunatum* plant which is known to have many uses customarily for various medications. It is also a well-known ethno medicinal plant amongst the Mizo tribe of Mizoram state. The phytochemical analysis revealed that various secondary metabolites such as saponin, flavonoid, phenol, alkaloid and cardiac glycosides were present (Table 1). Previous research reported that phenolics, steroids, di- and triterpenes, flavonoids, volatile oils are present in this genus[44]. It is reported that plant phytochemical contents ensured the medicinal properties such as anti-cancer, anti-inflammatory, antioxidant activities[45,46].

The present study also showed that phenolics and flavonoids content were comparatively high whereas alkaloid is found to be lesser as compared to the other secondary metabolites (Fig. 1). The phenols possess high antioxidant capacity and are an important dietary additive of plants. It has been reported that the scavenging effect of the DPPH free radical significantly elevated with the increase in concentration of the sample and standard to a certain degree and hence are strongly

reliant on the extract concentration[47-49]. Phenolics have been a centre of attraction as potential agents to prevent and treat many oxidative stress-related diseases[50-52]. The flavonoids have a major contribution for the attractive colours of flowers with anthocyanins[53,54]. The antioxidant activity depends mostly on the secondary metabolites found in the plants. It expresses the amount of antioxidant needed to neutralize the unstable free radicals in the system[55]. The assessment of the ability to scavenge DPPH is a very useful method to estimate the antioxidant activity[56]. It is a violet coloured compound and has an unpaired electron in the outermost shell which turns yellow after accepting electron from any antioxidant[57]. The high antioxidant properties might be due to the presence of high flavonoid and phenol present in the leaf extract of *C. infortunatum* (Fig. 2).

The toxicity studies of *C. infortunatum* against HeLa, AGS and HT-29 cells exhibited an increase in cytotoxicity with the rise in concentration (Fig.3). It was reported that methanolic extract of *C. serratum* leaves reduced tumour development in 7,12-dimethylbenz[α]anthracene (DMBA) induced skin carcinogenicity in testis, liver and kidney of mice[58-59]. Previous report stated that the methanolic extract of *Clerodendrum serratum* illustrates good *in vivo* activity using Dalton's Lymphoma Ascites[60]. Other plants containing flavonoids such as *Oroxylum indicum*, *Tragopogon porrifolius*, *Trigonella foenum-graecum*, *Cassia acutifolia* and *Rhazya stricta* have also been shown to have antioxidant and anticancer activities[61-63].

The gas chromatography-mass spectrometry analyses indicated the occurrence of 14 bioactive volatile compounds. The gas chromatographic study reported the presence of bioactive compounds such as hispidulin-glucuronide, eupafolin, scutellarin, 2-acetoxyclerodendrin B and hispidulin 7-O-glucuronide from *C. infortunatum*[64]. These chemicals, however, were not found in present experiment; it may be because of geographical as well as ecological and plant growth factors[42]. The detection of neophytadiene (diterpene) in our study (Fig. 4) which is histamine release inhibitor is a noble compound with the activities of antioxidants, analgesic, anti-inflammatory and antimicrobial[65,66]. Further, the presence of oleic acid, octadecatrienoic acid, Phenol,4-(-methylpropyl) were already reported and discussed in the previous studies of *C. infortunatum* leaves extract[67]. The present study indicated that benzofuran, 2,3-dihydro is compound of dihydro derivative of benzofuran which were used as anticancer agents and inhibitors of NF-κB[68].

In previous report, Waliullah *et al*. reported that the ethanol leaf extract of *C. infortunatum* has shown noteworthy inhibitory potency against the fungal and bacterial strain by testing out the MIC and its zone of inhibition[69]. Similarly, Saha *et al.* also testified that the chloroform extract of *C. infortunatum* has high content of phenol and flavonoids and it is effective against *Phomopsis vexans* and the antifungal activity was also proven by Kharkwal *et al*[70,71]. In addition to that, there were also studies that *C. infortunatum* possessed a substantial antitumor activity against DLA cells *in vitro* as well as EAC cell lines *in vivo*[72,73]. The presence of rutin and quercetin detected using HPLC (Fig. 5) which has been shown to have anti-cancer activity both *in vitro* and *in vivo*[74,75]. Rutin is able to curb the expression of Bax and Bcl-2 which eventually triggers different caspase enzymes leading to apoptosis in colon cancer cells[76] and can also reduce AP-1 and NF-κB transactivation significantly[77]. Quercetin has the capacity to induce DNA interaction, cell cycle arrest as well as activating apoptosis in different cancer cells[78,79]. Hence, rutin and quercetin along with benzofuran identified using HPLC and GC-MS could be the reason for the anti-cancer activity observed in our study.

Conclusion

Clerodendrum infortunatum contain various phytochemicals and exhibits significant anti-oxidant activity leading to cell cytotoxicity against HeLa, HT-29 and AGS, respectively. Therefore, disseminating the awareness about the significance of *C. infortunatun* is required which is a known ethnomedicinal plant in traditional system of Mizo tribe for its unique medicinal values especially in rural areas. Parallely, the conservation measures also needs to be taken into consideration for this plant. Moreover, the mechanism of action might be essential to study at molecular level for dosage formulation in future. Our study revealed that the anti-tumor activity of *C. infortunatum* could be possibly due to the flavonoids rutin and quercetin as well as benzofuran. Besides, our study also validates the plant's traditional use to treat a variety of diseases by the Mizo ethnic tribe. Hence, the *C. infortunatum* could be a viable candidate for more research as anti-cancer agent.

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Conflict of interest

All authors declare no conflict of interest.

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